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Annex D

Sequence of a cDNA coding for human glutathione peroxidase confirms TGA encodes active site selenocysteine

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Selenium dependent glutathione peroxidase (1) is a nuclear encoded cytosolic and mitochondrial enzyme which maintains the integrity of DNA and lipids as well as reducing levels of endogenous hydrogen peroxide as follows:

$$ROOH + 2\,GSH \rightarrow GSSG + ROH + H_2O$$

where ROOH represents peroxidized DNA (2), lipid hydroperoxides, membrane-associated phospholipid hydroperoxides or hydrogen peroxide. We have isolated a cDNA coding the human enzyme from a kidney library in λgt10 by cross-hybridization with a bovine cDNA (3); 24 of 5300 clones hybridized with the probe. The active site selenocysteine residue (-CH_2SeH) at position 47 (i.e. SeC) is encoded by the nonsense codon, TGA, as is similarly observed in the mouse gene (4). Interestingly evidence suggests that the selenium atom is incorporated cotranslationally (5) rather than via a posttranslational modification step. This clone possesses 5 bp of the 5'-untranslated region, the 603 bp coding region, 223 bp of the 3'-untranslated region and a canonical polyadenylation signal, AATAAA, upstream of the polyA tract. The amino acid sequence reveals the protein possesses approximately 87% and 85% homology with preprocessed bovine (3) and mouse enzymes, respectively.

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          1                   10                  20
        Met Cys Ala Ala Arg Leu Ala Ala Ala Ala Gln Ser Val Tyr Ala Phe Ser Ala Arg Pro Leu Ala Gly Gly Glu Pro Val
GCCCC   ATG TGT GCT GCT CGC CTA GCG GCC GCC GCC GCG CAG TCG GTG TAT GCC TTC TCG GCC CGC CCG CTG GCC GGC GGG GAG CCT GTC    89

     30                  40                  50
Ser Leu Gly Ser Leu Arg Gly Lys Val Leu Leu Ile Glu Asn Val Ala Ser Leu SeC Gly Thr Thr Val Arg Asp Tyr Thr Gln Met Asn
AGC CTG GGC TCC GTG CGG GGC AAG GTA CTA CTT ATC GAG AAT GTG GCC TCC CTC TGA GGG ACC ACC GTC CGG GAC TAC ACC CAG ATG AAC   179

     60                  70                  80
Glu Leu Gln Arg Arg Leu Gly Pro Arg Gly Leu Val Val Leu Gly Phe Pro Cys Asn Gln Phe Gly His Gln Glu Asn Ala Lys Asn Glu
GAG CTG CAG CGC CGC CTC GGA CCC CGG GGC CTG GTG GTC CTC GGC TTC CCG TGC AAC CAG TTT GGG CAT CAG GAG AAC GCC AAG AAC GAA   269

     90                  100                 110
Glu Ile Gln Asn Ser Leu Lys Tyr Val Arg Pro Gly Gly Gly Phe Glu Pro Asn Phe Met Leu Phe Glu Lys Cys Glu Val Asn Gly Ala
GAG ATT CAG AAT TCC CTC AAG TAC GTC CGC CCT GGT GGT GGG TTC GAG CCC AAC TTC ATG CTC TTC GAG AAG TCC GAG GTG AAC GGT GCG   359

     120                 130                 140
Gly Ala His Pro Leu Phe Ala Phe Leu Arg Glu Ala Leu Pro Ala Pro Ser Asp Asp Ala Thr Ala Leu Met Thr Asp Pro Lys Leu Ile
GGC GCG CAC CCT CTC TTC GCC TTC CTG CGG GAG GCC CTC CCA GCT CCC ACC GAC GAC GCG ACC GCG CTT ATG ACC GAC CCC AAG CTC ATC   449

     150                 160                 170
Thr Trp Ser Pro Val Cys Arg Asn Asp Val Ala Trp Asn Phe Glu Lys Phe Leu Val Gly Pro Asp Gly Val Pro Leu Arg Arg Tyr Ser
ACC TGG TCT CCC GTG TGT CGC AAC GAT GTT GCC TGG AAC TTT GAG AAG TTC CTG GTC GGC CCT GAC GGT GTG CCC CTA CGC AGG TAC AGC   539

     180                 190                 200 201
Arg Arg Phe Gln Thr Ile Asp Ile Glu Pro Asp Ile Glu Ala Leu Leu Ser Gln Gly Pro Ser Cys Ala AM
CGC CGC TTC CAG ACC ATT GAC ATC GAG CCT GAC ATC GAA GCC CTG CTG TCT CAA GGG CCC AGC TGT GCC TAG CGCGCCCCTCCTACCCCCGCCTGCT 635

TGCCAGTTGCACTGCTGCTGTCTCGGGCCGGCTTTTCATCTATGAGGGTGTTTCCTCTAAACGTACGAGGGACGAACACCTGATCTTACAGAAAATACCAACCTCGAGATGGCTGCTGGTCC 755

TGTTGATCCCACTTCTCTGCCAGACCAAGCCCAGTTTCCCCACTAATAAAGTGCCCGGTGTCAGCAAAAAAAAAAAAA                                          832

Translated Mol. Weight = 21964.60
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Nucleotide sequence of cDNA for rabbit glutathione peroxidase

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The cDNA coding rabbit glutathione peroxidase was isolated from liver cDNA library in lambda gt11 by cross hybridization with the rat glutathione peroxidase cDNA which was cloned in this laboratory and reported elsewhere(1). The cDNA consisted of 600 bp of the coding region and the nucleotide sequence revealed that TGA, which is to be the stop codon in general, encoded seleno-cysteine(SeC) residue as was proved to be so with glutathione peroxidases of mouse (2), man(3) and rat(1). The amino acid sequence deduced from cDNA possesses 84, 84, 87 and 85% homology with rat, mouse, human, and bovine(4) enzymes, respectively.

```
                                                                  cccgctttgtctcacacc
  1 ATGTGTGCGGCTCGTATGGCGGCGGCTGCCCAGTCTGTGTACTCCTTCTCAGCGCACCCGCTGGCCGGCGGGGAG
    M  C  A  A  R  M  A  A  A  A  Q  S  V  Y  S  F  S  A  H  P  L  A  G  G  E
 76 CCCGTGAACCTGGGCTCCCTGCGGGGCAAGGTGCTGCTCATTGAGAATGTGGCGTCGCTCTGAGGCACTACGGTC
    P  V  N  L  G  S  L  R  G  K  V  L  L  I  E  N  V  A  S  L [SeC] G  T  T  V
151 CGGGACTACACCCAGATGAACGAGCTGCAAGAGCGCCTCGGGCCCCGGGCCCTGGTCGTGCTCGGCTTCCCGTGC
    R  D  Y  T  Q  M  N  E  L  Q  E  R  L  G  P  R  A  L  V  V  L  G  F  P  C
226 AACCAGTTTGGGCATCAGGAGAACGCCAAGAATGAGGAGATTCTGAATTCCCTCAAGTATGTCCGGCCTGGAGGC
    N  Q  F  G  H  Q  E  N  A  K  N  E  E  I  L  N  S  L  K  Y  V  R  P  G  G
301 GGGTTCGAGCCCAACTTCATGCTCTTCCAGAAGTGCGAGGTGAACGGCGCCAAGGCCAGCCCGCTCTTCGCCTTC
    G  F  E  P  N  F  M  L  F  Q  K  C  E  V  N  G  A  K  A  S  P  L  F  A  F
376 CTGCGGGAGGCCCTGCCGCCGCCCAGCGACGACCCCACTGCGCTCATGACCGACCCCAAGTTCATCACCTGGTGC
    L  R  E  A  L  P  P  P  S  D  D  P  T  A  L  M  T  D  P  K  F  I  T  W  C
451 CCGGTGTGCCGTAACGACGTTTCCTGGAGCTTCGAGAAGTTCCTGGTGGGCCCCGATGGTGTTCCCGTGCGCAGG
    P  V  C  R  N  D  V  S  W  S  F  E  K  F  L  V  G  P  D  G  V  P  V  R  R
526 TACAGCCGCCGCTTCCCCACCATCGACATCGAGCCCGACATCCAAGCCCTGCTGTCCAAGGGGTCTGGCGGTGCC
    Y  S  R  R  F  P  T  I  D  I  E  P  D  I  Q  A  L  L  S  K  G  S  G  G  A
601 TAGggcgccccctaccctggctgcttgccagtggcctgctgctctctgggggtttcatccatgafffcgttcccc
    *
676 cgaaaacaaatggaggaacgcctgatgtccgggaaaccccccaggtgggcgctggtcctgtccatccc 742
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